

=> D L1 SQIDE 1-4

L1 ANSWER 1 OF 4 REGISTRY COPYRIGHT 2000 ACS
RN 252726-88-8 REGISTRY
CN Mannanase, endo-1,4-.beta.- (Bacillus strain I633) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 59: PN: WO9964619 SEQID: 2 claimed protein
FS PROTEIN SEQUENCE
SQL 460

→ does not have search report

SEQ 1 NANSIFYVSG TTLYDANGNP FVMRGINHG AWYKDQATTA IEGIANTGAN
=====

51	TVRIVLSDGG	QWTKDDIHTV	RNLISLAEDN	HLVAVPEVHD	ATGYDSIASL
101	NRAVDYWIEM	RSALIGKEDT	VIINIANEWF	GSWEGDAWAD	GYKQAIPLRL
151	NAGLNHTLMV	DAAGWGQFPQ	SIHDYGREVF	NADPQRNTMF	SIHMYEYAGG
201	NASQVRTNID	RVLNQDLALV	IGFEGHRHTN	GDVDEATIMS	YSEQRGVGWL
251	AWSWKNGNPE	WEYLDLSNDW	AGNNLTAWGN	TIVNGPYGLR	ETSRLSTVFT
301	GGGSDGGTSP	TTLYDFEGSM	QGWTGSSLSG	GPWAVTEWSS	KGSHSLKADI
351	QLSSNSQHYL	HVIQNTSLQQ	NSRIQATVKH	ANWGSVGNMG	TARLYVKTGH
401	GYTWYSGSFV	PINGSSGTTL	SLDLSNVQNL	SQVREIGVQF	QSASDSSGQT
451	SIYIDNVIVE				

HITS AT: 2-18
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS

1 REFERENCES IN FILE CA (1967 TO DATE)
1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L1 ANSWER 2 OF 4 REGISTRY COPYRIGHT 2000 ACS
RN 252726-85-5 REGISTRY
CN 1-300-Mannanase, endo-1,4-.beta.- (Bacillus strain I633) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 57: PN: WO9964619 SEQID: 2 claimed protein
FS PROTEIN SEQUENCE
SQL 300

SEQ 1 NANSIFYVSG TTLYDANGNP FVMRGINHG AWYKDQATTA IEGIANTGAN
=====

51	TVRIVLSDGG	QWTKDDIHTV	RNLISLAEDN	HLVAVPEVHD	ATGYDSIASL
101	NRAVDYWIEM	RSALIGKEDT	VIINIANEWF	GSWEGDAWAD	GYKQAIPLRL
151	NAGLNHTLMV	DAAGWGQFPQ	SIHDYGREVF	NADPQRNTMF	SIHMYEYAGG
201	NASQVRTNID	RVLNQDLALV	IGFEGHRHTN	GDVDEATIMS	YSEQRGVGWL
251	AWSWKNGNPE	WEYLDLSNDW	AGNNLTAWGN	TIVNGPYGLR	ETSRLSTVFT

HITS AT: 2-18
MF Unspecified
CI MAN
SR CA
LC STN Files: CA, CAPLUS

1 REFERENCES IN FILE CA (1967 TO DATE)
1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L1 ANSWER 3 OF 4 REGISTRY COPYRIGHT 2000 ACS
RN 252726-70-8 REGISTRY
CN Mannanase, endo-1,4-.beta.- (Bacillus strain I633 precursor) (9CI) (CA INDEX NAME)
OTHER NAMES:

CN 2: PN: WO9964619 SEQID: 2 claimed protein
FS PROTEIN SEQUENCE
SQL 490

SEQ 1 LNNGFKKIFS ITLSLLASS ILFVSGTSTA NANSIFYVSG TTYDANGNP
=====

51	FVMRGINHG	AWYKDQATTA	IEGIANTGAN	TVRIVLSDGG	QWTKDDIHTV
101	RNLISLAEDN	HLVAVPEVHD	ATGYDSIASL	NRAVDYWIEM	RSALIGKEDT
151	VIINIANEWF	GSWEGDAWAD	GYKQAIPLR	NAGLNHTLMV	DAAGWGQFPQ
201	SIHDYGREVF	NADPQRNTMF	SIHMYEYAGG	NASQVRTNID	RVLNQDLALV
251	IGIEFGHRHTN	GDVDEATIMS	YSEQRGVGWL	AWSWKNGNGPE	WEYLDLSNDW
301	AGNNLTAWGN	TIVNGPYGLR	ETSRLSTVFT	GGGSDGGTSP	TTYLDFEGSM
351	QGWGTGSSLSG	GPWAVTEWSS	KGSHSLKADI	QLSSNSQHYL	HVIQNTSLQQ
401	NSRIQATVKH	ANWGSVGNMG	TARLYVKTGH	GYTWYSGSFV	PINGSSGTTL
451	SLDLSNVQNL	SQVREIGVQF	QSASDSSGQT	SIYIDNVIVE	

HITS AT: 32-48

MF Unspecified

CI MAN

SR CA

LC STN Files: CA, CAPLUS

1 REFERENCES IN FILE CA (1967 TO DATE)

1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

L1 ANSWER 4 OF 4 REGISTRY COPYRIGHT 2000 ACS

RN 252726-46-8 REGISTRY

CN Mannanase, endo-1,4-.beta.- (Bacillus strain I633) fusion protein with
peptide (synthetic linker) fusion protein with cellulase (Clostridium
thermocellum strain YS gene cipB fragment) (9CI) (CA INDEX NAME)

OTHER NAMES:

CN 4: PN: WO9964619 SEQID: 4 claimed protein

FS PROTEIN SEQUENCE

SQL 476

SEQ 1 ANSGFYVSGT TTYDANGNPF VMRGINHGHA WYKDQATTAI EGIANTGANT
=====

51	VRIVLSDGGQ	WTKDDIHTVR	NLISLAEDNH	LVAVPEVHDA	TGYDSIASLN
101	RAVDYWIEMR	SALIGKEDTV	IINIANEWF	GSWEGDAWADG	YKQAIPLRN
151	AGLNHTLMVD	AAGWGQFPQS	IHDYGREVFN	ADPQRNTMFS	IHMYEYAGGN
201	ASQVRTNIDR	VLNQDLALVI	GEFGHRHTNG	DVDEATIMSY	SEQRGVGWLA
251	WSWKNGNGPEW	EYLDLSNDWA	GNNLTAWGNT	IVNGPYGLRE	TSRLSTVFTA
301	SPEPTPEPTA	NTPVSGNLKV	EFYNSNPSDT	TNSINPQFKV	TNTGSSAIDL
351	SKLTLRYYYT	VDGQKDQTFW	CDHAAIIGSN	GSYNGITSNV	KGTFVKMSSS
401	TNNADTYLEI	SFTGGTLEPG	AHVQIQGRFA	KNDWSNYTQS	NDYSFKRSRQ
451	FVEWDQVTAY	LNGVLVWGKE	PGGSVV		

HITS AT: 1-17

MF Unspecified

CI MAN

SR CA

LC STN Files: CA, CAPLUS

1 REFERENCES IN FILE CA (1967 TO DATE)

1 REFERENCES IN FILE CAPLUS (1967 TO DATE)

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

09/339,159

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12Q		A2	(11) International Publication Number: WO 99/64619
			(43) International Publication Date: 16 December 1999 (16.12.99)
(21) International Application Number: PCT/DK99/00314		(72) Inventors; and	
(22) International Filing Date: 10 June 1999 (10.06.99)		(75) Inventors/Applicants (for US only): KAUPPINEN, Markus, Sakari [FI/DK]; Egegade 10,5, DK-2200 Copenhagen N. (DK). SCHÜLEIN, Martin [DK/DK]; Wiedeweltsgade 51, DK-2100 Copenhagen Ø (DK). SCHNORR, Kirk [US/DK]; Nørrebrogade 44A, 1. tv., DK-2200 Copenhagen N. (DK). ANDERSEN, Lene, Nonboe [DK/DK]; Laksevej 11, DK-3450 Allerød (DK). BJØRNVAAD, Mads, Eskelund [DK/DK]; Dr. Abildgaards Allé 8, 3th, DK-1955 Frederiksberg (DK).	
(30) Priority Data:		(74) Common Representative: NOVO NORDISK A/S; Corporate K-2880 Bagsværd (DK).	
09/111,256	10 June 1998 (10.06.98) US	<p>AL, AM, AT, AU, AZ, BA, BB, BG, BI, BR, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LS, LT, LU, LV, MD, MG, MK, MN, MU, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, SM, SN, ST, SV, TH, TJ, TM, TR, UA, UG, US, UZ, VN, YU, ZA, ZW, ZY, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p>	
PA 1998 01341	20 October 1998 (20.10.98) DK		
PA 1998 01340	20 October 1998 (20.10.98) DK		
60/105,970	28 October 1998 (28.10.98) US		
60/106,054	28 October 1998 (28.10.98) US		
PA 1998 01725	23 December 1998 (23.12.98) DK		
PA 1999 00308	5 May 1999		
PA 1999 00307	5 May 1999		
PA 1999 00306	5 May 1999		
PA 1999 00309	5 May 1999		
60/123,543	9 May 1999	<p>Published Without international search report and to be republished upon receipt of that report. With an indication in relation to deposited biological material furnished under Rule 13bis separately from the description.</p>	
60/123,641	10 May 1999		
60/123,623	10 May 1999		
60/123,642	11 May 1999		
(71) Applicant (for all designated states except US): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).			
(54) Title: NOVEL MANNANASES			
<p>BaiSpec5, <i>Bacillus clausii</i> NN017546 BaiAlcal, <i>Bacillus alcalophilus</i> BaiSpec3, <i>Bacillus pseudoalcalophilus</i> BaiSpor2, <i>Bacillus sporothermodurans</i></p>			
0.10			
(57) Abstract			
<p>Novel mannanases comprising e.g. an amino acid sequence as shown in positions 31-330 of SEQ ID NO: 2 or their homologues may be derived from e.g. <i>Bacillus</i> sp. 1633, or may be encoded by polynucleotide molecules comprising a nucleotide sequence as shown in SEQ ID NO: 1 from nucleotide 91 to nucleotide 990, polynucleotide molecules that encode a polypeptide that is at least 65 % identical to the amino acid sequence of SEQ ID NO: 2 from amino acid residue 31 to amino acid residue 330, or degenerate nucleotide sequences thereof. The mannanases are alkaline and are useful e.g. in cleaning compositions, in a fracturing fluid useful to fracture a subterranean formation, for modifying plant material, and for treatment of cellulosic fibres.</p>			

CLAIMS

1. An isolated mannanase which is
 - (a) a polypeptide encodable by the mannanase enzyme encoding
5 part of the DNA sequence cloned into the plasmid present in
Escherichia coli DSM 12197, or
 - (b) a polypeptide comprising an amino acid sequence as shown in
positions 31-330 of SEQ ID NO:2, or
 - (c) a polypeptide encodable by the DNA sequence as shown in
10 positions 91-990 or positions 91-1470 of SEQ ID NO:1, or
 - (d) an analogue of the polypeptide defined in (a) or (b) which
is at least 65% homologous with said polypeptide, or a fragment
of (a), (b) or (c).
- 15 2. The mannanase according to claim 1 which is derivable from a
strain of *Bacillus sp.*
3. The mannanase according to claim 2 which has
 - i) a relative mannanase activity of at least 60% in the pH range
20 7.5-10, measured at 40°C;
 - ii) a molecular weight of 34 ± 10 kDa, as determined by SDS-PAGE;
and/or
 - iii) the N-terminal sequence ANSGFYVSGTTLYDANG.
- 25 4. An isolated polynucleotide molecule comprising a DNA sequence
encoding an enzyme exhibiting mannanase activity, which DNA
sequence comprises:
 - (a) the mannanase encoding part of the DNA sequence cloned into
the plasmid present in *Escherichia coli* DSM 12197;
 - 30 (b) the DNA sequence shown in positions 91-1470 in SEQ ID NO 1,
preferably position 91-990, or its complementary strand;

- (c) an analogue of the DNA sequence defined in (a) or (b) which is at least 65% homologous with said DNA sequence;
- (d) a DNA sequence which hybridizes with a double-stranded DNA probe comprising the sequence shown in positions 91-990 in SEQ ID NO 1 at low stringency;
- (e) a DNA sequence which, because of the degeneracy of the genetic code, does not hybridize with the sequences of (b) or (d), but which codes for a polypeptide having exactly the same amino acid sequence as the polypeptide encoded by any of these DNA sequences; or
- a DNA sequence which is a fragment of the DNA sequences specified in (a), (b), (c), (d), or (e).

5. The cloned DNA sequence according to claim 4, in which the DNA sequence encoding an enzyme exhibiting mannanase activity is obtained from a microorganism, preferably a filamentous fungus, a yeast, or a bacteria; preferably from *Bacillus*, *Caldicellulosiruptor* or *Humicola*.

6. An isolated polynucleotide molecule encoding a polypeptide having mannanase activity which polynucleotide molecule hybridizes to a denatured double-stranded DNA probe under medium stringency conditions; wherein the probe is selected from the group consisting of DNA probes comprising the sequence shown in positions 91-990 of SEQ ID NO:1, the sequence shown in positions 91-1470 of SEQ ID NO:1 and DNA probes comprising a subsequence of positions 91-990 of SEQ ID NO:1 having a length of at least about 100 base pairs.

7. An expression vector comprising the following operably linked elements: a transcription promoter; a DNA segment selected from the group consisting of (a) polynucleotide molecules encoding a

polypeptide having mannanase activity comprising a nucleotide sequence as shown in SEQ ID NO:1 from nucleotide 91 to nucleotide 990, (b) polynucleotide molecules encoding a polypeptide having mannanase activity that is at least 65% identical to the amino acid sequence of SEQ ID NO:2 from amino acid residue 31 to amino acid residue 330, and (c) degenerate nucleotide sequences of (a) or (b); and a transcription terminator.

8. A cultured cell into which has been introduced an expression vector according to claim 7, wherein said cell expresses the polypeptide encoded by the DNA segment.

9. An isolated polypeptide having mannanase activity selected from the group consisting of:

- 15 (a) polypeptide molecules comprising an amino acid sequence as shown in SEQ ID NO: 2 from residue 31 to residue 330; and
(b) polypeptide molecules that are at least 65% identical to the amino acids of SEQ ID NO: 2 from amino acid residue 31 to amino acid residue 330.

20

10. The polypeptide according to claim 9 which is produced by *Bacillus* sp. I633.

11. An enzyme preparation comprising a purified polypeptide according to claim 9.

12. A method of producing a polypeptide having mannanase activity comprising culturing a cell into which has been introduced an expression vector according to claim 7, whereby said cell expresses a polypeptide encoded by the DNA segment; and recovering the polypeptide.

13. The preparation according to claim 11 which further comprises one or more enzymes selected from the group consisting of proteases, cellulases (endoglucanases), β -glucanases, hemicellulases, lipases, peroxidases, laccases, α -amylases, glucoamylases, cutinases, pectinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, pectate lyases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, pectin lyases, other mannanases, pectin methylesterases, cellobiohydrolases, transglutaminases; or mixtures thereof.

14. An isolated enzyme having mannanase activity, in which the enzyme is (i) free from homologous impurities, and (ii) produced by the method according to claim 12.

15. A method for improving the properties of cellulosic or synthetic fibres, yarn, woven or non-woven fabric in which method the fibres, yarn or fabric is treated with an effective amount of the preparation according to claim 11 or an effective amount of the enzyme according to claim 1 or 2.

16. The method according to claim 15, wherein the enzyme preparation or the enzyme is used in a desizing process step.

25

17. A method for degradation or modification of plant material in which method the plant material is treated with an effective amount of the preparation according to claim 11 or an effective amount of the enzyme according to claim 1 or 2.

30

18. The method according to claim 17 wherein the plant material is recycled waste paper; mechanical, chemical, semichemical,

kraft or other paper-making pulps; fibres subjected to a retting process; or guar gum or locust bean gum containing material.

19. A method for processing liquid coffee extract, in which
5 method the coffee extract is treated with an effective amount of the preparation according to claim 11 or an effective amount of the enzyme according to claim 1 or 2.

20. A cleaning composition comprising the enzyme preparation
10 according to claim 11 or the enzyme according to claim 1 or 2.

21. The cleaning composition according to claim 20 which further comprises an enzyme selected from cellulases, proteases, lipases, amylases, pectin degrading enzymes and xyloglucanases;
15 and conventional detergent ingredient.

22. The cleaning composition according to claim 20 wherein said enzyme or enzyme preparation is present at a level of from 0.0001% to 2%, preferably from 0.0005% to 0.5%, more preferably
20 from 0.001% to 0.1% pure enzyme by weight of total composition.

23. The cleaning composition according to claim 21 wherein the enzyme is present at a level of from 0.0001% to 2%, preferably from 0.0005% to 0.5%, more preferably from 0.001% to 0.1% pure
25 enzyme by weight of total composition.

24. The cleaning composition according to claim 21 wherein the enzyme is an amylase.

30 25. The cleaning composition according to claim 24 which further comprises yet another enzyme selected from cellulase, protease, lipase, pectin degrading enzyme and xyloglucanase.

26. The cleaning composition according to claim 21 which comprises a surfactant selected from anionic, nonionic, cationic surfactant, and/or mixtures thereof.

5

27. The cleaning composition according to claim 21 which comprises a bleaching agent.

28. The cleaning composition according to claim 21 which comprises a builder.

29. A fabric softening composition according to claim 21 which comprises a cationic surfactant comprising two long chain lengths.

15

30. A process for machine treatment of fabrics which process comprises treating fabric during a washing cycle of a machine washing process with a washing solution containing the enzyme preparation according to claim 11 or the enzyme according to claim 1 or 2.

31. Use of the enzyme preparation according to claim 11 or the enzyme according to claim 1 or 2 together with a enzyme selected from cellulase, protease, lipase, amylase, pectin degrading enzyme and xyloglucanase in a cleaning composition for fabric cleaning and/or fabric stain removal.

32. Use of the enzyme preparation according to claim 11 or the enzyme according to claim 1 or 2 together with a enzyme selected from cellulase, amylase, protease, lipase, pectin degrading enzyme and xyloglucanase in a cleaning composition for cleaning hard surfaces such as floors, walls, bathroom tile and the like.

33. Use of the enzyme preparation according to claim 11 or the enzyme according to claim 1 or 2 together with a enzyme selected from cellulase, amylase, protease, lipase, pectin degrading
5 enzyme and xyloglucanase in a cleaning composition for hand and machine dishwashing.

34. Use of the enzyme preparation according to claim 11 or the enzyme according to claim 1 or 2 together with a enzyme selected
10 from cellulase, amylase, protease, lipase, pectin degrading enzyme and/or xyloglucanase in a cleaning composition for oral, dental, contact lenses and personal cleaning applications.

35. An isolated mannanase which is
15 (a1) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in *Escherichia coli* DSM 12180, or
(b1) a polypeptide comprising an amino acid sequence as shown in positions 32-344 of SEQ ID NO:6, or
20 (c1) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment of (a1), (b1) or (c1);
(a2) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in
25 *Escherichia coli* DSM 12433, or
(b2) a polypeptide comprising an amino acid sequence as shown in positions 32-362 of SEQ ID NO:10, or
(c2) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment
30 of (a2), (b2) or (c2);
(a3) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in

Escherichia coli DSM 12441, or

(b3) a polypeptide comprising an amino acid sequence as shown in positions 33-331 of SEQ ID NO:12, or

(c3) an analogue of the polypeptide defined in (a) or (b) which
5 is at least 85% homologous with said polypeptide, or a fragment of (a3), (b3) or (c3);

(a4) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in
Escherichia coli DSM 9984, or

10 (b4) a polypeptide comprising an amino acid sequence as shown in positions 166-488 of SEQ ID NO:14, or

(c4) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment of (a4), (b4) or (c4);

15 (a5) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in
Escherichia coli DSM 12432, or

(b5) a polypeptide comprising an amino acid sequence as shown in positions 68-369 of SEQ ID NO:16, or

20 (c5) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment of (a5), (b5) or (c5);

(a6) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in

25 *Escherichia coli* DSM 12849, or

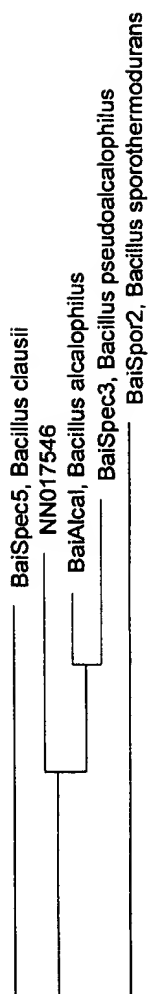
(b6) a polypeptide comprising an amino acid sequence as shown in positions 29-320 of SEQ ID NO:22, or

(c6) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment
30 of (a6), (b6) or (c6);

(a7) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in

- Escherichia coli DSM 12180, or
- (b7) a polypeptide comprising an amino acid sequence as shown in positions 301-625 of SEQ ID NO:26, or
- (c7) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment of (a7), (b7) or (c7);
- (a8) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in Escherichia coli DSM 12851, or
- 10 (b8) a polypeptide comprising an amino acid sequence as shown in positions 166-496 of SEQ ID NO:28, or
- (c8) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment of (a8), (b8) or (c8);
- 15 (a9) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in Escherichia coli DSM 12852, or
- (b9) a polypeptide comprising an amino acid sequence as shown in positions 26-361 of SEQ ID NO:30, or
- 20 (c9) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment of (a9), (b9) or (c9);
- (a10) a polypeptide encoded by the mannanase enzyme encoding part of the DNA sequence cloned into the plasmid present in
- 25 Escherichia coli DSM 12436, or
- (b10) a polypeptide comprising an amino acid sequence as shown in positions 593-903 of SEQ ID NO:32, or
- (c10) an analogue of the polypeptide defined in (a) or (b) which is at least 85% homologous with said polypeptide, or a fragment
- 30 of (a10), (b10) or (c10).

1/3



0.10

Fig. 1